Evidence for intracellular bacterial activity in deeply frozen saline ice formations

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Direct evidence for metabolism in a variety of frozen environments has pushed temperature limits for bacterial activity to increasingly lower temperatures (so far to –20°C). To date, the metabolic activities of marine psychrophilic bacteria, important components of sea-ice communities, have not been studied in laboratory culture at such low temperatures. In this study, we measured [^{3}H]-leucine incorporation into macromolecules, further fractionated biochemically, by the marine psychrophilic bacterium *Colwellia psychrerythraea* strain 34H (suspended in artificial seawater) over a range of anticipated activity-permissive temperatures, from +13°C to –20°C, including expected negative controls at –80 and –196°C.. We also examined the effect of extracellular polymeric substances (EPS) on [^{3}H] leucine incorporation. Results showed that live cells of strain 34H incorporated substantial amounts of [^{3}H] leucine into TCA-precipitable material (primarily into protein) down to –20°C, with rates enhanced by EPS at subzero temperatures and no activity detected in the killed controls for strain 34H (or in *E. coli* controls), which included TCA-killed, heat-killed, sodium azide and chloramphenicol treated samples. Surprisingly, evidence for low but significant rates of intracellular incorporation of [3H]-leucine into protein was also obtained for samples incubated at –80 and –196°C. How cells can incorporate leucine into proteins at such low temperatures needs to be examined further, but the process of vitrification promoted by deep-freezing, salts and organic polymers may be relevant. This work suggests that a distinction between intracellular and

extracellular processes and their relative contributions to microbial metabolic activity at very low temperatures needs to be examined in more detail.